

Ampelisca Abdita & Nereis Virens
Sediment Toxicity Tests
of the
Calcasieu Estuary, Louisiana



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INTRODUCTION

The acute toxicity of sediments from the Calcasieu Estuary located in southwest Louisiana was assessed as a measure of the biological effects of sediment-associated contaminants. Whole sediment toxicity and bioaccumulation tests were conducted at the Harding ESE, Inc. Toxicology Laboratory. The test organism used for the whole sediment toxicity tests was the euryhaline benthic amphipod, *Ampelisca abdita*. Sediment bioaccumulation tests were conducted with the polychaete, *Nereis virens* to determine the bioavailability of sediment-associated contaminants.

The 10-day whole sediment tests were conducted following the procedures outlined in the US Environmental Protection Agency “Methods for Assessing the Toxicity of Sediment-associated Contaminants with Estuarine and Marine Amphipods” (EPA/600/R-025; USEPA 1994). The 28-day bioaccumulation tests were conducted in accordance with the U.S. Environmental Protection Agency and the U.S. Army Corps of Engineers Standard Testing Manual entitled: *Evaluation of Dredged Material for Proposed Ocean Disposal - Testing Manual* (USEPA-503/8-91/001, February, 1991). All of the original raw data pertaining to these studies are maintained at Harding ESE, Inc., 404 SW 140th Terrace, Newberry, Florida 32669-3000.

METHODS

Sample Collection, Log-In, and Holding

Sediments were collected during November 3 through December 2, 2000 from the coastal waters off southwest Louisiana (Figure 1 through 6) by CDM personnel and packed into pre-cleaned 1-gallon polyethylene wide mouth bottles for transport. Wet ice was used to preserve the samples in coolers during shipment. The shipments arrived between November 7 and December 5, 2000 (Table 1). The deliveries to Harding ESE Toxicology Laboratory were shipped by priority overnight courier service. Upon arrival, samples were inspected to determine their condition (e.g., lids in place or leakage) and chain-of-custody information was reviewed to determine proper sample identification. Samples identified as 00BI2-ST001-008,010-031,041-NSD-010, 00BV2-ST001-010-NSD-010, 00SN2-ST001-006,008-015,035,038, 00LC2-ST001-014,027-NSD-010, 00UC2-ST001,003-005,007-008,010-031,037-NSD-010 were received from November 8, 2000 through December 4, 2000 (Table 1). A broken jar was noted for Sample 00BI2-ST005-NSD-010 upon sample receipt at Harding ESE on November 8, 2000; the remaining samples were received in good condition. CDM personnel collected a second sample of 00BI2-ST005-NSD-010, which was received intact at the Harding ESE laboratory on November 16, 2000. The transport temperature of the samples was measured and recorded in the Sediment Receipt Logbook maintained at the Harding ESE Toxicology Laboratory. Chain-of-custody pages were placed with Harding ESE Toxicology Laboratory test data in experiment binders and project files. Chain-of-custody and other traffic information pertaining to the test samples is presented in Appendix A. All samples were stored in a refrigerator in the dark at $4 \pm 2^{\circ}\text{C}$ until testing.

Control Sediment

The control sediment used for the *A. abdita* whole sediment tests, was collected by the organism supplier, Breezina and Associates. The control sediment used in the *N. virens* bioaccumulation tests was collected by the test organism supplier, Aquatic Research Organisms, Hampton, New Hampshire. The two organism suppliers collected the control sediments at low tide from the site locations from which the test organisms were collected. Each of these control sediments have been used in the past by the organism suppliers for culturing and holding test organisms and has been proven to be suitable for these purposes. The control sediments were shipped in 1-gallon plastic jugs from each of the suppliers, with cool packs in insulated containers to the Harding ESE Toxicology Laboratory (Table 1).

Overlying Water

The overlying water used for the sediment tests was natural filtered seawater, collected at Marineland, Florida. Salinity of the filtered seawater was adjusted by dilution with well water obtained from a deep well located at Harding ESE to the appropriate salinity for each of the test types (28 ppt for *A. abdita* and 25 ppt for *N. virens*).

Organism Collection and Holding

Ampelisca abdita

Ampelisca abdita (2-4mm) were purchased from Breezina and Associates, P.O. Box 25, Dillon Beach, California 94929. The amphipods were shipped in 1-gallon plastic jugs, with cool packs in insulated containers to the Harding ESE Toxicology Laboratory. Amphipods were held in the laboratory in pre-sieved, uncontaminated sediment (obtained from the organism supplier) from the collection site, under static conditions with light aeration as recommended by the organism supplier.

Nereis virens

Nereis virens (3-15grams) The polychaete, *N. virens*, used in the bioaccumulation study were obtained from Aquatic Research Organisms, Hampton, New Hampshire. The polychaetes were shipped in insulated containers with cool packs to the Harding ESE Toxicology Laboratory. The organisms were held in the shipping containers at test temperature until the bioaccumulation tests were initiated.

Sediment Preparation

Prior to use in testing, the sediment samples were thoroughly homogenized within the original shipping containers and hand processed to remove stones, plant debris, and indigenous organisms. Sediment samples not easily hand processed, were press sieved through a 1.0-mm mesh stainless-steel screen. Any observations made during the sediment preparation process were recorded on a daily log sheet.

Test Apparatus and Conditions

Ampelisca abdita

Amphipods were exposed to test sediments for 10-days under static conditions, following ASTM procedures (ASTM 1995). Harding ESE initiated the sediment tests on two different days: 50 samples on November 17, 2000 and 50 samples on December 8, 2000. Test chambers were 10.5-cm (width) by 23-cm (height) glass jars. Two hundred milliliters of prepared sediment was added to each of five replicate chambers per sample. Eight hundred milliliters of naturally filtered seawater (28 ppt) was added as overlying water. All test chambers were numbered and individual numbers randomly assigned to jars. Test chambers were arranged in a 20°C water bath in ascending order by jar number to ensure that the replicates for each treatment were randomly placed within the water bath.

A turbine regeneration air plant provided oil-free air that was delivered into the water column via a 1-ml pipette inserted into each test chamber. The aeration provided acceptable dissolved oxygen concentrations (>60% saturation) throughout the study period. Ambient laboratory lighting was continuous during the 10-day test to inhibit swimming behavior of the organisms and promote tube-dwelling activity. Tests sediment and overlying water was added to test vessels on the day before the start of the test, assuring that test sediments were well settled before the addition of test organisms. Salinity of the overlying water was checked on Day 0 of the study period. Salinity for each of the test exposures was within the appropriate range (28 ± 2 ppt) (Appendix B). Overlying water was renewed prior to test initiation and the addition of the test organisms.

At the beginning of the test, amphipods were sieved from holding containers through a 0.5-mm mesh stainless-steel screen, rinsed with seawater, and collected from the water with a plastic pipette. Twenty sub-adult amphipods were randomly distributed into plastic weigh boats. The condition and number of organisms was independently verified prior to actual loading of the organisms into test vessels. Organism loading into the test vessels was performed in a random order with a tape slip placed on each “loaded” test vessel. After one hour, the chambers were examined for any amphipods that had not burrowed into the sediment. Organisms that had not burrowed in the one-hour period were replaced. The amphipods were not fed during the test period.

Test chambers were monitored daily and the number of animals found on the sediment surface, in the water column, or on the water surface as recorded. Dead amphipods were removed and noted in the daily log and the organism survival sheet. Live, emerged amphipods trapped on the water surface were gently prodded with a stream of overlying water applied with a plastic pipette and allowed to reburrow.

Water quality parameters (temperature, salinity, dissolved oxygen (DO), and pH) were monitored daily throughout the test in a different replicate for each sample. Temperature was determined using a temperature probe, coupled to a VWR Digital Thermometer. Salinity was measured with a hand-held portable refractometer. DO was measured with a YSI DO meter (Model 55), and pH was measured with an Orion pH meter (Model 290A) and Orion Triode pH probe. All readings were observed with

instrument probes below the surface of the overlying water and in close proximity to the sediment surfaces. All instruments and equipment were calibrated daily prior to use and operated according to the manufacturer's specifications and Harding ESE standard operating procedures (SOPs).

Overlying water and pore water samples were analyzed for total ammonia. Overlying water samples were collected twice during the test period (i.e., on days 1 and 9). A composite sample was obtained by removing approximately 15 mLs from each replicate test chamber for a particular sample. Sediment pore water samples were prepared for analysis by mixing on a 1 to 1 ratio with the dilution water and aspiration from the remaining sediment. Ammonia samples were analyzed for total ammonia (mg/L) to a detection limit of 0.1 mg/L using an Orion meter (Model-SA 290A) with an Orion Model 95-12 ammonia probe.

The sediment tests were terminated at the end of the 10-day study period. The test sediments were washed, with seawater, through a 0.5-mm stainless-steel screen. All material remaining on the screen was rinsed into a dish labeled with the appropriate sample replicate. The contents of each dish were sorted and living organisms were removed and placed in a plastic weigh boat with seawater and counted. All samples for which greater than 10% (e.g., 2 out of 20) of the original organisms were unaccounted for were reexamined. Amphipods not accounted for at test termination were assumed dead and recorded as such.

Nereis virens

The bioaccumulation tests were performed for 28 days (from December 14, 2000 through January 11, 2001) using five replicates of each site sediment and the laboratory control without dilution. Each replicate test chamber was a 10-gallon aquarium to which 2 centimeters of sediment were added. Each of the exposure aquaria was filled with approximately 8 gallons natural seawater with a salinity of 25 ± 2 ppt. The test chambers were allowed to settle overnight and twenty *N. virens* were then added to each test chamber on Day 0 of the study period (December 14, 2000). Test organisms were not fed at any time during the testing period.

The tests were performed in a temperature-controlled room adjusted to maintain a constant test temperature of $18 \pm 2^\circ\text{C}$, and under laboratory illumination (~ 1050 Lux). Aeration was provided to all the test chambers at approximately 100-120 bubbles per minute with the aid of an oil-free laboratory air compressor (Aquatic Eco Systems, Inc., Clearwater, Florida).

Water quality parameters measured daily during the 28-day sediment bioaccumulation tests were D.O., pH, temperature, and salinity. A quantitative measurement of water quality parameters was not obtained on Christmas Day and New Year's Day (December 25, 2000 and January 1, 2001, respectively) due to the holiday constraints. Dissolved oxygen was measured with a YSI Model 55 DO meter, temperature was measured with a VWR thermocouple, pH was measured with an Orion Model SA 290A pH meter, and salinity was measured with an Aquatic Eco Systems CL893 refractometer. All instruments were calibrated daily before use. Observations were made daily for

organism behavior and mortality. Survival counts were performed at test termination for all of the bioaccumulation tests.

Renewals of the overlying water in the aquaria were performed three times per week. Water was siphoned from the aquaria through 11/16-inch (outside diameter) Tygon® tubing and the aquaria were refilled with seawater pumped from a holding tank through similar tubing. The Tygon® tubing, equipped with plastic pinch clamps and tipped with plastic T-joints, was connected to PVC pipes fitted with control valves to adjust the flow of water. Care was taken to ensure that the sediment in each tank was not disturbed during renewals.

After 28 days of exposure, test organisms from each replicate were removed from the aquaria and allowed to depurate in clean seawater for 24 hours. After depuration, organisms from each replicate were rinsed in deionized water, placed into glass sample jars, and stored in a freezer at $-10 \pm 2^{\circ}\text{C}$. Frozen *N. virens* tissues were shipped to American Analytical and Technical Services, Inc., for chemical analyses on January 16, 2001.

Reference Toxicant Test

A reference toxicant water-only test with sodium dodecyl sulfate (SDS) was conducted with *A. abdita*. Only one reference toxicant test was performed because all sediment tests (100 samples) were initiated within 30 days of each other. Ninety-six hour survival was determined in each concentration test chamber to which ten test organisms had been added. A dilution series of seven concentrations ranging between 0.0 (control seawater) and 20.00 mg/L was used. Trimmed Spearman-Kärber Method (Version 1.5) was used to calculate an LC50 for the reference toxicant test (Gulley, 1994; Hamilton, 1977).

RESULTS

Test Conditions

Test conditions, including lighting, ammonia, alkalinity, pH, DO, and temperature remained at acceptable levels throughout the testing period for all test series. Copies of the relevant raw data pertaining to the bioassay tests are provided in Appendices B and E. The two sets of *A. abdita* tests, one for each of the 50 samples, were conducted November 17 through November 27, 2000 and December 8 through December 18, 2000. The *N. virens* bioaccumulation tests were conducted December 14, 2000 through January 11, 2002. All sediment tests were initiated within 20 days of sample receipt. Test organisms were held in the laboratory <4 days.

Water Quality

Ampelisca abdita

Water quality parameters (pH, dissolved oxygen, and salinity) were measured daily in the overlying waters of one replicate per sample per day (Appendix B). Test conditions including temperature, pH, and salinity remained within acceptable conditions throughout the test period. A measurement of 4.6 mg/L DO was noted on Day 0 of the test, therefore aeration was initiated at a rate of approximately 60 bubbles per minute to

maintain DO levels above 60 percent. Dissolved oxygen (DO) ranged between 4.6 and 8.4 mg/L for the test period (Appendix B).

Ammonia was measured in overlying water of the replicate test chambers near the beginning and end of the test period (Appendix B). Total ammonia in the overlying water of the test chambers ranged from < 0.1 to 0.4 mg/L for the initial samples analyzed, and between 0.1 and 0.5 mg/L for the final total ammonia analyses. The total ammonia no observable effect concentration (NOEC) is 30 mg/L at pH 7.7 (USEPA, 1994). The NOEC for total ammonia was not exceeded during the test period for any overlying water sample. Total ammonia in the sediment pore water samples ranged from 0.1 to 1.0 mg/L. The NOEC for total ammonia was not exceeded during the test period for any sediment pore water sample (Appendix B)

Survival

The test organism survival data for the sediment tests are presented in Table 2 and Appendix B. Mean performance control survival was 92 percent for the Control group (Controls 1 through 4). The survival data are summarized by sample in Table 2. After 10-days exposure to test sediments, survival of *A. abdita* ranged from 0 percent (sample stations 00BI2-ST014-NSD-010, 00BI2-ST016-NSD-010, 00BI2-ST017-NSD-010, 00BI2-ST018-NSD-010, 00BI2-ST024-NSD-010, 00BI2-ST026-NSD-010, 00BV2-ST001-NSD-010, 00BV2-ST002-NSD-010, 00BV2-ST003-NSD-010, 00BV2-ST004-NSD-010, 00BV2-ST005-NSD-010, 00BV2-ST008-NSD-010, 00LC2-ST005-NSD-010, 00LC2-ST006-NSD-010, 00UC2-ST028-NSD-010) to 91 percent at sample station 00SN2-ST011-NSD-010.

Nereis virens

The bioaccumulation test conditions, including temperature, DO, pH, and salinity, were maintained at acceptable levels throughout the 28-day testing period. The laboratory raw data is provided in Appendix E.

Survival

Data for the survival of *N. virens* in the bioaccumulation tests are presented in Table 4. *N. virens* survivorship in the laboratory control sediment was 99%. Survival of *N. virens* in the site sediments ranged from 87% (sample station 00BI2-ST030-NSD-010) to 98% (sample station 00UC2-ST028-NSD-010) (Table 4). Adequate mass of *N. virens* tissue was available for chemical analyses for all samples and tissue samples were shipped to American Analytical and Technical Services, Inc. on January 16, 2001.

Reference Toxicant Tests

The 96-hour LC₅₀ for the *A. abdita* reference toxicant test was calculated to be 1.69 mg/L SDS. The results from the reference toxicant test are within current control limits at Harding ESE and demonstrated that the test species was within the expected sensitivity range. Harding ESE infrequently uses *A. abdita* for testing purposes and has a limited amount of data from which to develop a control chart. The results from the reference toxicant test used in this study are presented in Appendix D along with Harding ESE historical reference toxicant data for *A. abdita* and a control chart developed using the historical data.

CONCLUSIONS

After 10 days of exposure, several of the sediment samples had 0 percent survival for *A. abdita*. Organism survival in sediment from sample stations 00BI2-ST014-NSD-010, 00BI2-ST016-NSD-010, 00BI2-ST017-NSD-010, 00BI2-ST018-NSD-010, 00BI2-ST024-NSD-010, 00BI2-ST026-NSD-010, 00BV2-ST001-NSD-010, 00BV2-ST002-NSD-010, 00BV2-ST003-NSD-010, 00BV2-ST004-NSD-010, 00BV2-ST005-NSD-010, 00BV2-ST008-NSD-010, 00LC2-ST005-NSD-010, 00LC2-ST006-NSD-010, 00UC2-ST028-NSD-010 was 0 percent. The highest percent survival was observed sample station 00SN2-ST011-NSD-010 sediment at 91 percent. Water quality parameters remained within suitable limits for the sediment toxicity tests.

REFERENCES

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U.S. Environmental Protection Agency (EPA). 1988. *Computer Program and Users Guide for Probit and Dunnett's Analysis of Data from Acute and Short Term Chronic Toxicity Tests with Aquatic Organisms*. Prepared by Statistical Support Staff, Computer Sciences Corporation. Prepared for the Biological Methods Branch, Environmental Monitoring and Support Laboratory, Cincinnati, OH, 1988.

Table 1. Tracking for Calcasieu Estuary Sediment Samples used to Conduct Sediment Tests.

Sample ID	Date Arrived	Date Shipped	Date Collected	Test Start	Test End	Days Held
Control 1	11/15/00	11/14/00	11/13/00	11/17/00	12/18/00	2
Control 2	11/15/00	11/14/00	11/13/00	11/17/00	12/18/00	2
Control 3	12/06/00	12/05/00	11/04/00	12/08/00	12/18/00	2
Control 4	12/06/00	12/05/00	11/04/00	12/08/00	12/18/00	2
Control *	* 12/12/00	* 12/11/00	* 12/10/00	* 12/14/00	* 01/11/01	* 2
00BI2-ST001-NSD-010	11/08/00	11/06/00	11/05/00	11/17/00	11/27/00	9
00BI2-ST002-NSD-010	11/08/00	11/06/00	11/05/00	11/17/00	11/27/00	9
00BI2-ST003-NSD-010	11/08/00	11/06/00	11/05/00	11/17/00	11/27/00	9
00BI2-ST004-NSD-010	11/08/00	11/07/00	11/06/00	* 11/17/00	* 11/27/00	9
00BI2-ST005-NSD-010	11/16/00	11/15/00	11/14/00	* 11/17/00	* 11/27/00	1
00BI2-ST006-NSD-010	11/08/00	11/06/00	11/05/00	11/17/00	11/27/00	9
00BI2-ST007-NSD-010	12/05/00	12/04/00	12/02/00	12/08/00	12/18/00	3
00BI2-ST008-NSD-010	11/08/00	11/07/00	11/06/00	11/17/00	11/27/00	9
00BI2-ST010-NSD-010	11/08/00	11/06/00	11/04/00	11/17/00	11/27/00	9
00BI2-ST011-NSD-010	11/13/00	11/11/00	11/10/00	* 11/17/00	* 11/27/00	4
00BI2-ST012-NSD-010	11/13/00	11/11/00	11/10/00	11/17/00	11/27/00	4
00BI2-ST013-NSD-010	11/13/00	11/11/00	11/10/00	11/17/00	11/27/00	4
00BI2-ST014-NSD-010	11/14/00	11/13/00	11/10/00	11/17/00	11/27/00	3
00BI2-ST015-NSD-010	11/14/00	11/13/00	11/10/00	11/17/00	11/27/00	3
00BI2-ST016-NSD-010	11/14/00	11/13/00	11/10/00	11/17/00	11/27/00	3
00BI2-ST017-NSD-010	11/14/00	11/13/00	11/10/00	11/17/00	11/27/00	3
00BI2-ST018-NSD-010	11/13/00	11/11/00	11/10/00	11/17/00	11/27/00	4
00BI2-ST019-NSD-010	11/13/00	11/11/00	11/10/00	* 11/17/00	* 11/27/00	4
00BI2-ST020-NSD-010	11/13/00	11/11/00	11/10/00	11/17/00	11/27/00	4
00BI2-ST021-NSD-010	11/14/00	11/13/00	11/09/00	11/17/00	11/27/00	3
00BI2-ST022-NSD-010	11/14/00	11/13/00	11/10/00	11/17/00	11/27/00	3
00BI2-ST023-NSD-010	11/14/00	11/13/00	11/10/00	11/17/00	11/27/00	3
00BI2-ST024-NSD-010	11/14/00	11/13/00	11/10/00	11/17/00	11/27/00	3
00BI2-ST025-NSD-010	11/08/00	11/07/00	11/06/00	11/17/00	11/27/00	9
00BI2-ST026-NSD-010	11/14/00	11/13/00	11/10/00	11/17/00	11/27/00	3
00BI2-ST027-NSD-010	11/15/00	11/14/00	11/12/00	* 12/08/00	*12/18/00	23
00BI2-ST028-NSD-010	12/04/00	12/02/00	12/01/00	12/08/00	12/18/00	4
00BI2-ST029-NSD-010	12/04/00	12/02/00	12/01/00	12/08/00	12/18/00	4
00BI2-ST030-NSD-010	12/04/00	12/02/00	12/01/00	* 12/08/00	* 12/18/00	4
00BI2-ST031-NSD-010	12/04/00	12/02/00	12/01/00	12/08/00	12/18/00	4
00BI2-ST041-NSD-010	11/08/00	11/07/00	11/06/00	12/08/00	12/18/00	30
00BV2-ST001-NSD-010	11/15/00	11/14/00	11/11/00	12/08/00	12/18/00	23
00BV2-ST002-NSD-010	11/15/00	11/14/00	11/11/00	12/08/00	12/18/00	23
00BV2-ST003-NSD-010	11/16/00	11/15/00	11/14/00	12/08/00	12/18/00	22
00BV2-ST004-NSD-010	11/15/00	11/14/00	11/11/00	12/08/00	12/18/00	23
00BV2-ST005-NSD-010	12/01/00	11/30/00	11/29/00	* 12/08/00	* 12/18/00	7
00BV2-ST006-NSD-010	11/15/00	11/14/00	11/11/00	12/08/00	12/18/00	23
00BV2-ST007-NSD-010	12/01/00	11/30/00	11/29/00	12/08/00	12/18/00	7
00BV2-ST008-NSD-010	11/16/00	11/15/00	11/14/00	12/08/00	12/18/00	22
00BV2-ST009-NSD-010	12/01/00	11/30/00	11/29/00	12/08/00	12/18/00	7
00BV2-ST010-NSD-010	12/01/00	11/30/00	11/29/00	12/08/00	12/18/00	7
00SN2-ST001-NSD-010	12/06/00	12/05/00	12/02/00	12/08/00	12/18/00	2
00SN2-ST002-NSD-010	12/06/00	12/05/00	12/02/00	12/08/00	12/18/00	2
00SN2-ST003-NSD-010	12/05/00	12/04/00	12/02/00	12/08/00	12/18/00	3
00SN2-ST004-NSD-010	12/05/00	12/04/00	12/02/00	12/08/00	12/18/00	3
00SN2-ST005-NSD-010	12/05/00	12/04/00	12/02/00	12/08/00	12/18/00	3
00SN2-ST006-NSD-010	11/09/00	11/08/00	11/07/00	* 11/17/00	* 11/27/00	8
00SN2-ST008-NSD-010	11/09/00	11/08/00	11/07/00	11/17/00	11/27/00	8

Table 1. Tracking for Calcasieu Estuary Sediment Samples used to Conduct Sediment Tests.

00SN2-ST009-NSD-010	11/09/00	11/08/00	11/07/00	12/08/00	12/18/00	29
00SN2-ST011-NSD-010	12/04/00	12/02/00	11/30/00	* 12/08/00	* 12/18/00	4
00SN2-ST012-NSD-010	12/04/00	12/02/00	12/01/00	12/08/00	12/18/00	4
00SN2-ST013-NSD-010	12/04/00	12/02/00	11/30/00	12/08/00	12/18/00	4
00SN2-ST014-NSD-010	12/04/00	12/02/00	12/01/00	12/08/00	12/18/00	4
00SN2-ST015-NSD-010	12/04/00	12/02/00	11/30/00	12/08/00	12/18/00	4
00SN2-ST035-NSD-010	11/09/00	11/08/00	11/07/00	12/08/00	12/18/00	29
00SN2-ST038-NSD-010	11/09/00	11/08/00	11/07/00	12/08/00	12/18/00	29
00LC2-ST001-NSD-010	12/01/00	11/30/00	11/29/00	12/08/00	12/18/00	7
00LC2-ST002-NSD-010	11/15/00	11/14/00	11/12/00	12/08/00	12/18/00	23
00LC2-ST003-NSD-010	11/08/00	11/06/00	11/04/00	11/17/00	11/27/00	9
00LC2-ST004-NSD-010	11/15/00	11/14/00	11/12/00	12/08/00	12/18/00	23
00LC2-ST005-NSD-010	11/16/00	11/15/00	11/14/00	12/08/00	12/18/00	22
00LC2-ST006-NSD-010	11/16/00	11/15/00	11/14/00	* 12/08/00	* 12/18/00	22
00LC2-ST007-NSD-010	11/16/00	11/15/00	11/14/00	12/08/00	12/18/00	22
00LC2-ST008-NSD-010	12/04/00	12/02/00	12/01/00	12/08/00	12/18/00	4
00LC2-ST009-NSD-010	11/15/00	11/14/00	11/13/00	11/17/00	11/27/00	2
00LC2-ST010-NSD-010	12/04/00	12/02/00	11/30/00	12/08/00	12/18/00	4
00LC2-ST011-NSD-010	12/04/00	12/02/00	11/30/00	12/08/00	12/18/00	4
00LC2-ST012-NSD-010	12/04/00	12/02/00	11/30/00	12/08/00	12/18/00	4
00LC2-ST013-NSD-010	11/15/00	11/14/00	11/13/00	12/08/00	12/18/00	23
00LC2-ST014-NSD-010	11/16/00	11/14/00	11/13/00	12/08/00	12/18/00	22
00LC2-ST027-NSD-010	11/15/00	11/14/00	11/12/00	12/08/00	12/18/00	23
00UC2-ST001-NSD-010	11/10/00	11/09/00	11/08/00	11/17/00	11/27/00	7
00UC2-ST003-NSD-010	11/15/00	11/14/00	11/12/00	12/08/00	12/18/00	23
00UC2-ST004-NSD-010	11/14/00	11/13/00	11/12/00	11/17/00	11/27/00	3
00UC2-ST005-NSD-010	11/13/00	11/11/00	11/09/00	11/17/00	11/27/00	4
00UC2-ST007-NSD-010	11/13/00	11/11/00	11/09/00	11/17/00	11/27/00	4
00UC2-ST008-NSD-010	11/09/00	11/06/00	11/04/00	11/17/00	11/27/00	8
00UC2-ST010-NSD-010	11/08/00	11/07/00	11/06/00	11/17/00	11/27/00	9
00UC2-ST011-NSD-010	11/08/00	11/07/00	11/06/00	* 11/17/00	* 11/27/00	9
00UC2-ST012-NSD-010	11/08/00	11/07/00	11/06/00	11/17/00	11/27/00	9
00UC2-ST013-NSD-010	11/14/00	11/13/00	11/09/00	11/17/00	11/27/00	3
00UC2-ST014-NSD-010	11/16/00	11/15/00	11/14/00	12/08/00	12/18/00	22
00UC2-ST015-NSD-010	11/09/00	11/06/00	11/03/00	11/17/00	11/27/00	8
00UC2-ST016-NSD-010	11/14/00	11/13/00	11/09/00	11/17/00	11/27/00	4
00UC2-ST017-NSD-010	11/10/00	11/09/00	11/08/00	11/17/00	11/27/00	7
00UC2-ST018-NSD-010	11/10/00	11/09/00	11/08/00	11/17/00	11/27/00	7
00UC2-ST019-NSD-010	11/14/00	11/13/00	11/09/00	11/17/00	11/27/00	3
00UC2-ST020-NSD-010	11/15/00	11/14/00	11/09/00	12/08/00	12/18/00	23
00UC2-ST021-NSD-010	11/10/00	11/09/00	11/08/00	11/17/00	11/27/00	7
00UC2-ST022-NSD-010	11/15/00	11/14/00	11/09/00	12/08/00	12/18/00	23
00UC2-ST023-NSD-010	11/13/00	11/11/00	11/09/00	11/17/00	11/27/00	4
00UC2-ST024-NSD-010	11/13/00	11/11/00	11/09/00	11/17/00	11/27/00	4
00UC2-ST025-NSD-010	11/13/00	11/11/00	11/09/00	11/17/00	11/27/00	4
00UC2-ST026-NSD-010	11/10/00	11/09/00	11/08/00	11/17/00	11/27/00	7
00UC2-ST027-NSD-010	11/13/00	11/11/00	11/09/00	11/17/00	11/27/00	4
00UC2-ST028-NSD-010	11/15/00	11/14/00	11/11/00	* 12/08/00	* 12/18/00	23
00UC2-ST029-NSD-010	11/14/00	11/13/00	11/09/00	11/17/00	11/27/00	3
00UC2-ST030-NSD-010	12/01/00	11/30/00	11/29/00	12/08/00	12/18/00	7
00UC2-ST031-NSD-010	12/01/00	11/30/00	11/29/00	12/08/00	12/18/00	7
00UC2-ST037-NSD-010	12/04/00	12/02/00	12/01/00	12/08/00	12/18/00	4

* indicates that the sample was also used for the *N. virens* bioaccumulation tests.

Source: ESE, 2001

Table 2. Survival of *Ampelisca abdita* Exposed to Whole Sediments From the Calcasieu Estuary, Louisiana during a 10-Day Toxicity Test. (Page 1 of 3)

Sample ID	Survival Replicate ^a					Survival (Percent) ^b
	A	B	C	D	E	
Control 1	19	18	16	18	19	90
Control 2	17	19	18	20	18	92
Control 3	19	19	19	20	19	96
Control 4	16	20	18	18	19	91
00BI2-ST001-NSD-010	11	9	11	9	11	51
00BI2-ST002-NSD-010	12	7	6	8	12	45
00BI2-ST003-NSD-010	5	5	4	3	4	21
00BI2-ST004-NSD-010	8	7	9	12	15	51
00BI2-ST005-NSD-010	9	10	5	3	8	35
00BI2-ST006-NSD-010	4	5	9	4	5	27
00BI2-ST007-NSD-010	12	8	13	9	11	53
00BI2-ST008-NSD-010	12	14	6	7	13	52
00BI2-ST010-NSD-010	15	17	10	15	10	67
00BI2-ST011-NSD-010	5	10	9	5	8	37
00BI2-ST012-NSD-010	8	5	3	0	7	23
00BI2-ST013-NSD-010	0	0	0	7	0	7
00BI2-ST014-NSD-010	0	0	0	0	0	0
00BI2-ST015-NSD-010	7	3	7	5	7	29
00BI2-ST016-NSD-010	0	0	0	0	0	0
00BI2-ST017-NSD-010	0	0	0	0	0	0
00BI2-ST018-NSD-010	0	0	0	0	0	0
00BI2-ST019-NSD-010	2	0	3	0	5	10
00BI2-ST020-NSD-010	4	6	6	15	10	41
00BI2-ST021-NSD-010	5	11	2	3	9	30
00BI2-ST022-NSD-010	14	12	6	15	5	52
00BI2-ST023-NSD-010	11	9	10	10	11	51
00BI2-ST024-NSD-010	0	0	0	0	0	0
00BI2-ST025-NSD-010	5	5	4	6	2	22
00BI2-ST026-NSD-010	0	0	0	0	0	0
00BI2-ST027-NSD-010	2	4	2	3	3	14
00BI2-ST028-NSD-010	11	17	18	11	6	63
00BI2-ST029-NSD-010	0	1	0	0	0	1
00BI2-ST030-NSD-010	1	0	0	0	2	3
00BI2-ST031-NSD-010	18	14	11	12	12	67
00BI2-ST041-NSD-010	13	7	12	14	8	54
00BV2-ST001-NSD-010	0	0	0	0	0	0
00BV2-ST002-NSD-010	0	0	0	0	0	0
00BV2-ST003-NSD-010	0	0	0	0	0	0
00BV2-ST004-NSD-010	0	0	0	0	0	0
00BV2-ST005-NSD-010	0	0	0	0	0	0
00BV2-ST006-NSD-010	1	0	1	0	0	2
00BV2-ST007-NSD-010	9	14	12	10	13	58
00BV2-ST008-NSD-010	0	0	0	0	0	0
00BV2-ST009-NSD-010	12	10	15	15	11	63

^a Twenty organisms exposed per replicate

^b Based on a total of 100 organisms per sample

Source: Harding ESE 2001

Table 2. Survival of *Ampelisca abdita* Exposed to Whole Sediments From the Calcasieu Estuary, Louisiana during a 10-Day Toxicity Test. (Page 2 of 3)

Sample ID	Survival Replicate ^a					Survival (Percent) ^b
	A	B	C	D	E	
00BV2-ST010-NSD-010	13	10	8	15	15	61
00SN2-ST001-NSD-010	12	12	17	16	10	67
00SN2-ST002-NSD-010	10	15	11	16	14	66
00SN2-ST003-NSD-010	14	15	19	12	19	79
00SN2-ST004-NSD-010	16	16	17	20	16	85
00SN2-ST005-NSD-010	14	16	20	17	16	83
00SN2-ST006-NSD-010	17	16	14	14	14	75
00SN2-ST008-NSD-010	15	15	16	15	15	76
00SN2-ST009-NSD-010	15	16	14	20	15	80
00SN2-ST011-NSD-010	17	19	16	20	19	91
00SN2-ST012-NSD-010	10	14	19	15	13	71
00SN2-ST013-NSD-010	10	13	15	15	16	69
00SN2-ST014-NSD-010	14	16	13	17	17	77
00SN2-ST015-NSD-010	16	15	18	17	11	77
00SN2-ST035-NSD-010	15	16	17	16	18	82
00SN2-ST038-NSD-010	16	16	12	19	19	82
00LC2-ST001-NSD-010	19	12	17	20	17	85
00LC2-ST002-NSD-010	9	8	14	10	12	53
00LC2-ST003-NSD-010	3	6	7	7	6	29
00LC2-ST004-NSD-010	14	15	12	11	14	66
00LC2-ST005-NSD-010	0	0	0	0	0	0
00LC2-ST006-NSD-010	0	0	0	0	0	0
00LC2-ST007-NSD-010	0	0	0	2	0	2
00LC2-ST008-NSD-010	12	14	16	9	16	67
00LC2-ST009-NSD-010	14	15	14	15	9	67
00LC2-ST010-NSD-010	8	12	10	13	12	55
00LC2-ST011-NSD-010	14	9	15	12	10	60
00LC2-ST012-NSD-010	14	13	15	14	16	72
00LC2-ST013-NSD-010	14	20	17	20	14	85
00LC2-ST014-NSD-010	11	9	10	14	9	53
00LC2-ST027-NSD-010	18	20	18	19	18	93
00UC2-ST001-NSD-010	17	12	15	10	10	64
00UC2-ST003-NSD-010	17	17	14	14	9	71
00UC2-ST004-NSD-010	12	11	9	12	8	52
00UC2-ST005-NSD-010	11	6	8	5	6	36
00UC2-ST007-NSD-010	9	3	6	10	3	31
00UC2-ST008-NSD-010	10	11	9	5	10	45
00UC2-ST010-NSD-010	12	12	12	6	11	53
00UC2-ST011-NSD-010	9	8	13	10	12	52
00UC2-ST012-NSD-010	8	2	8	4	6	28
00UC2-ST013-NSD-010	9	10	12	16	10	57
00UC2-ST014-NSD-010	15	13	15	11	12	66
00UC2-ST015-NSD-010	14	12	18	12	19	75
00UC2-ST016-NSD-010	15	14	13	11	17	70

^a Twenty organisms exposed per replicate

^b Based on a total of 100 organisms per sample

Source: Harding ESE 2001

Table 2. Survival of *Ampelisca abdita* Exposed to Whole Sediments From the Calcasieu Estuary, Louisiana during a 10-Day Toxicity Test. (Page 3 of 3)

Sample ID	Survival Replicate ^a					Survival (Percent) ^b
	A	B	C	D	E	
00UC2-ST017-NSD-010	18	14	11	12	10	65
00UC2-ST018-NSD-010	17	18	13	10	7	65
00UC2-ST019-NSD-010	11	15	14	14	12	66
00UC2-ST020-NSD-010	14	15	11	9	14	63
00UC2-ST021-NSD-010	9	13	12	9	11	54
00UC2-ST022-NSD-010	6	1	7	6	6	26
00UC2-ST023-NSD-010	16	16	14	10	13	69
00UC2-ST024-NSD-010	10	17	9	20	15	71
00UC2-ST025-NSD-010	13	8	12	7	7	47
00UC2-ST026-NSD-010	13	9	16	8	14	60
00UC2-ST027-NSD-010	17	5	8	11	16	57
00UC2-ST028-NSD-010	0	0	0	0	0	0
00UC2-ST029-NSD-010	7	10	9	6	5	37
00UC2-ST030-NSD-010	18	13	16	15	13	75
00UC2-ST031-NSD-010	11	16	13	18	14	72
00UC2-ST037-NSD-010	9	5	9	10	5	38

a Twenty organisms exposed per replicate

b Based on a total of 100 organisms per sample

Source: Harding ESE 2001

Table 4. Survival of *Nereis virens* Exposed to Sediments from the Calcasieu Estuary Area, Louisiana During a 28-day Bioaccumulation Test (Page 1 of 2).

Sample ID	Replicate	Number Alive and Percent Survival
Control	A	20
	B	20
	C	20
	D	19
	E	<u>20</u>
		99%
BI 030	A	19
	B	19
	C	15
	D	19
	E	<u>15</u>
		87%
SN 006	A	18
	B	17
	C	20
	D	17
	E	<u>18</u>
		90%
SN 011	A	18
	B	18
	C	20
	D	18
	E	<u>19</u>
		93%
BV 005	A	20
	B	19
	C	19
	D	20
	E	<u>19</u>
		97%
LC 006	A	16
	B	19
	C	17
	D	19
	E	<u>20</u>
		91%
BI 004	A	18
	B	18
	C	19
	D	16
	E	<u>20</u>
		91%
BI 005	A	17
	B	18
	C	18
	D	18
	E	<u>19</u>
		90%

Table 4. Survival of *Nereis virens* Exposed for 28 days to Sediments from the Calcasieu Estuary Area, Louisiana (Page 1 of 2).

Sample ID	Replicate	Number Alive and Percent Survival
UC 011	A	19
	B	18
	C	19
	D	17
	E	<u>20</u>
		93%
BI 019	A	18
	B	15
	C	19
	D	20
	E	<u>19</u>
		91%
BI 027	A	16
	B	20
	C	18
	D	20
	E	<u>19</u>
		93%
BI 011	A	17
	B	18
	C	20
	D	15
	E	<u>18</u>
		88%
UC 028	A	20
	B	20
	C	18
	D	20
	E	<u>20</u>
		98%

Source: Harding ESE, 2001

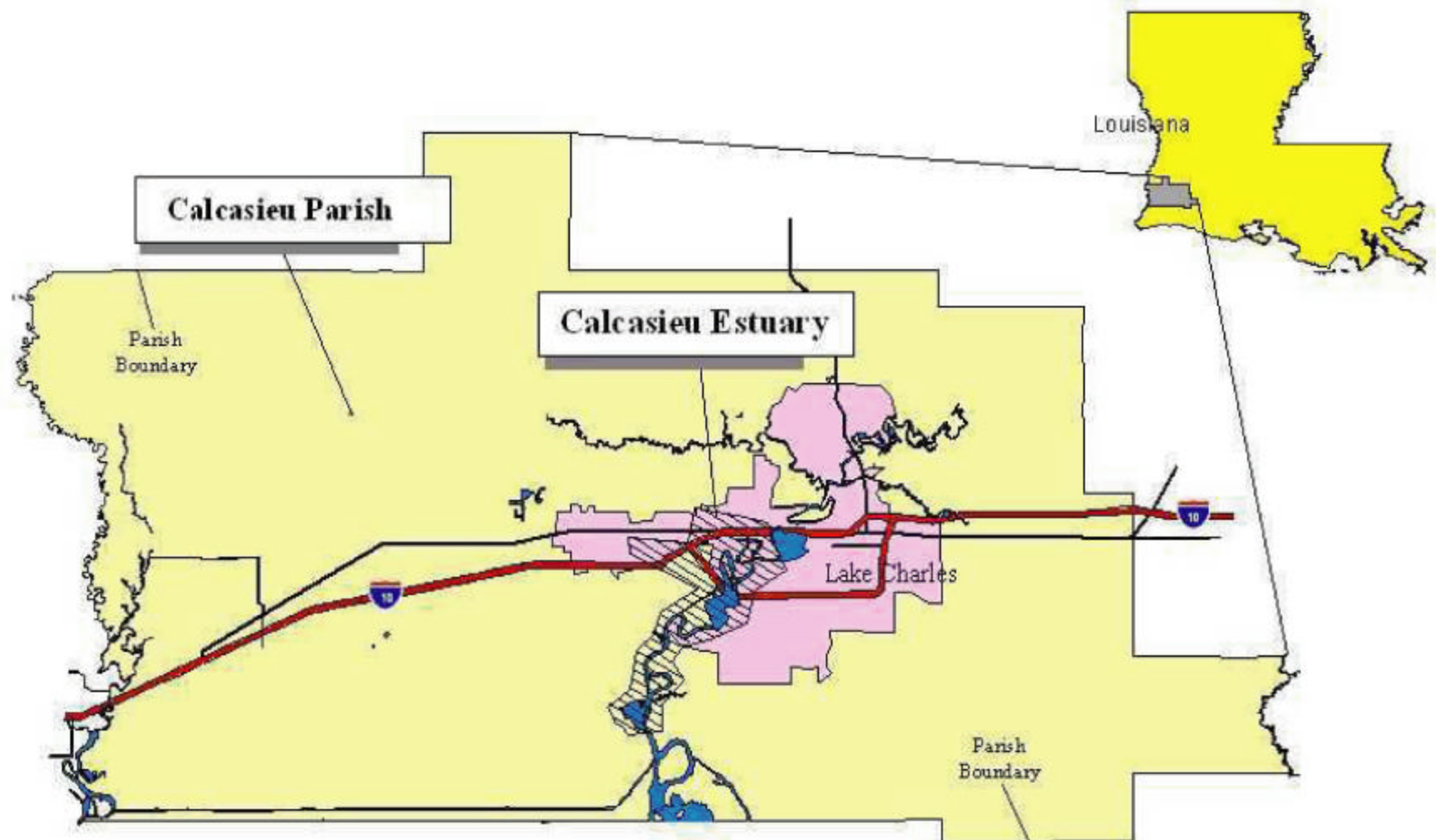




Figure 2
Bayou d'Inde
Sediment Quality Triad Locations



Figure 3
Upper Calcasieu & Bayou Verdine
Sediment Quality Triad Locations



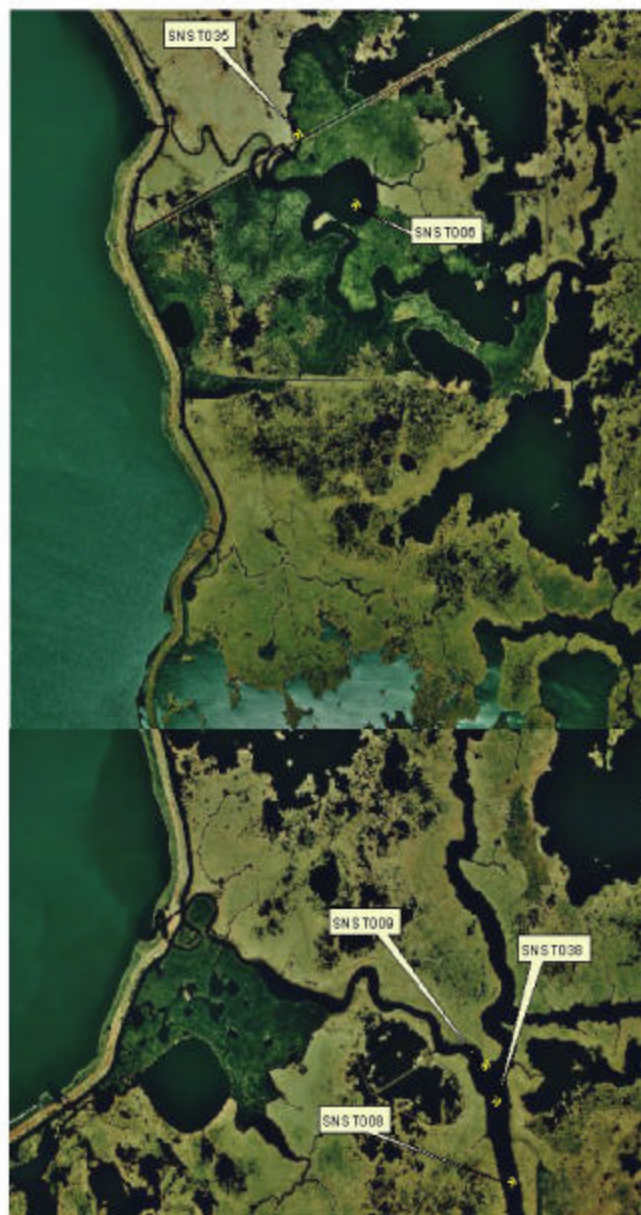
2000 0 2000 Meters

Figure 4
Lower Calcasieu
Sediment Quality Triad Locations



1000 0 1000 2000 Meters

Figure 5
Bayou Choupique
Sediment Quality Triad Locations



Bayou Bois Connine and Grand Bayou



Willow and Johnsons Bayou



0 3000 Meters

Figure 6
Bayou Bois Connine, Grand Bayou
Willow Bayou and Johnsons Bayou
Sediment Quality Triad Locations

Appendix A

Chain of Custody

Appendix B
10-Day *A. abdita* Sediment Toxicity Test Raw Data

Appendix C

Statistical Report

Appendix D

Reference Toxicant Test Data

Appendix E
28-day *Nereis virens* Bioaccumulation Test
Raw Data

Appendix F

Pore Water Data